PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 31/425, 31/395

A2

(11) International Publication Number: **WO 96/40128**

(43) International Publication Date: 19 December 1996 (19.12.96)

(21) International Application Number: PCT/US96/05464

(22) International Filing Date: 18 April 1996 (18.04.96)

(30) Priority Data:
08/477,493 7 June 1995 (07.06.95) US

(71) Applicant: THE SALK INSTITUTE FOR BIOLOGICAL STUDIES [US/US]; 10010 North Torrey Pines Road, La Jolla, CA 92037 (US).

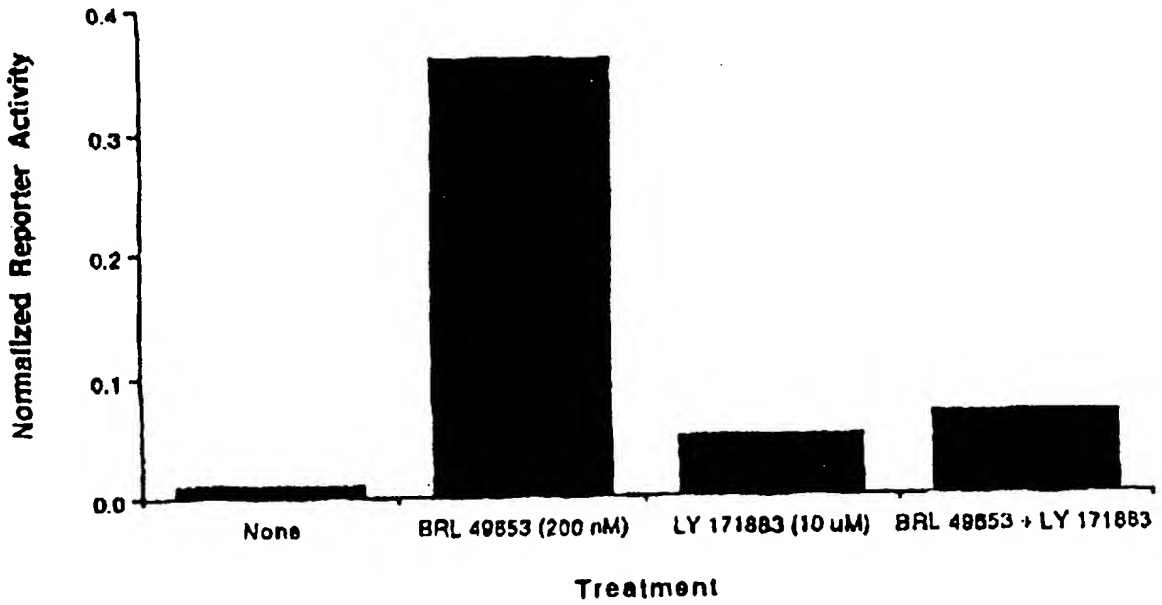
(72) Inventors: EVANS, Ronald, M.; 1471 Cottontail Lane, La Jolla, CA 92037 (US). FORMAN, Barry, M.; Apartment 299, 8568 Via La Jolla, La Jolla, CA 92037 (US).

(74) Agent: REITER, Stephen, E.; Pretty, Schroeder, Brueggemann & Clark, Suite 2000, 444 South Flower Street, Los Angeles, CA 90071 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: MODULATORS OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR-GAMMA, AND METHODS FOR THE USE THEREOF



(57) Abstract

In accordance with the present invention, there is provided a class of compounds which are capable of modulating processes mediated by peroxisome proliferator activated receptor-gamma (PPAR-γ). The identification of such compounds makes it possible to intervene in PPAR-γ mediated pathways.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

Modulators of Peroxisome Proliferator Activated Receptor-gamma, and Methods for the Use Thereof

FIELD OF THE INVENTION

The present invention relates to methods for the modulation of nuclear receptor mediated processes. In a particular aspect, the present invention relates to the use of a specific class of compounds for the modulation of processes mediated by peroxisome proliferator activated receptor-gamma (PPAR-γ).

BACKGROUND OF THE INVENTION

Peroxisome proliferators are a structurally diverse group of compounds which, when administered to rodents, elicit dramatic increases in the size and number of hepatic and renal peroxisomes, as well as concomitant increases in the capacity of peroxisomes to metabolize fatty acids via increased expression of the enzymes required for the ß-oxidation cycle (Lazarow and Fujiki, *Ann. Rev. Cell Biol.* 1:489-530 (1985); Vamecq and Draye, *Essays Biochem.* 24:1115-225 (1989); and Nelali et al., *Cancer Res.* 48:5316-5324 (1988)). Chemicals included in this group are the fibrate class of hypolipidermic drugs, herbicides, and phthalate plasticizers (Reddy and Lalwani, *Crit. Rev. Toxicol.* 12:1-58 (1983)). Peroxisome proliferation can also be elicited by dietary or physiological factors such as a high-fat diet and cold acclimatization.

Insight into the mechanism whereby peroxisome proliferators exert their pleiotropic effects was provided by the identification of a member of the nuclear hormone receptor superfamily activated by these chemicals (Isseman and Green, *Nature* 347-645-650 (1990)). This receptor, termed peroxisome proliferator activated receptor alpha

(PPARα), was subsequently shown to be activated by a variety of medium and long-chain fatty acids and to stimulate expression of the genes encoding rat acyl-CoA oxidase and hydratase-dehydrogenase (enzymes required for peroxisomal ß-oxidation), as well as rabbit cytochrome P450 4A6, a fatty acid ω-hydroxylase (Gottlicher et al., *Proc. Natl. Acad. Sci. USA* 89:4653-4657 (1992); Tugwood et al., *EMBO J.* 11:433-439 (1992); Bardot et al., *Biochem. Biophys. Res. Comm.* 192:37-45 (1993); Muerhoff et al., *J. Biol. Chem.* 267:19051-19053 (1992); and Marcus et al., *Proc. Natl. Acad. Sci. USA* 90(12):5723-5727 (1993).

The above-noted references suggest a physiological role for PPARα in the regulation of lipid metabolism. PPARα activates transcription by binding to DNA sequence elements, termed peroxisome proliferator response elements (PPRE), as a heterodimer with the retinoid X receptor. The retinoid X receptor is activated by 9-*cis* retinoic acid (see Kliewer et al., *Nature* 358:771-774 (1992), Gearing et al., *Proc. Natl. Acad. Sci. USA* 90:1440-1444 (1993), Keller et al., *Proc. Natl. Acad. Sci. USA* 90:2160-2164 (1993), Heyman et al., *Cell* 68:397-406 (1992), and Levin et al., *Nature* 355:359-361 (1992)). Since the PPARα-RXR complex can be activated by peroxisome proliferators and/or 9-*cis* retinoic acid, the retinoid and fatty acid signaling pathways are seen to converge in modulating lipid metabolism.

Since the discovery of PPARα, additional isoforms of PPAR have been identified, e.g., PPARβ, PPARγ and PPARδ, which are spatially differentially expressed. Because there are several isoforms of PPAR, it would be desirable to identify compounds which are capable of selectively interacting with only one of the PPAR isoforms. Such compounds would find a wide variety of uses, such as, for example, in the prevention of obesity, for the treatment of diabetes, and the like.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, we have identified a class of compounds which are capable of modulating processes mediated by peroxisome proliferator activated receptor-gamma (PPAR-γ). The identification of such compounds makes possible intervention in PPAR-γ mediated pathways.

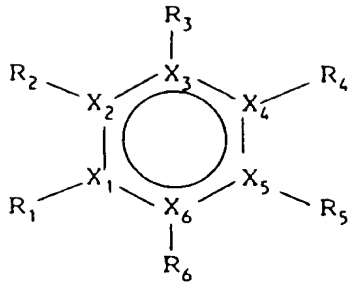
BRIEF DESCRIPTION OF THE FIGURE

Figure 1 illustrates the relative reporter activity induced by two different compounds when added alone or in combination to a GAL4-PPARγ fusion protein. In the figure, BRL 49653 refers to 5-[[4-[2-(methyl-2-pyridinylamino)ethoxy]phenyl]-methyl]-2,4-thiazolidenedione) and LY 171883 refers to 2-hydroxy-3-propyl-4-[6-(tetrazole-5-yl)butoxy]acetophenone.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided methods for modulating process(es) mediated by peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising conducting said process(es) in the presence of at least one antagonist or partial-agonist of PPAR-γ.

Antagonists and partial-agonists of PPAR contemplated for use in the practice of the present invention can be described broadly with reference to the general structure **I**:



(I)

wherein:

each of X_1, X_2, X_3, X_4, X_5 and X_6 is independently selected from carbon, nitrogen, oxygen or sulfur, with the proviso that at least three of the atoms forming the ring are carbon,

R_1 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, alkylaryl, substituted alkylaryl, alkenylaryl, substituted alkenylaryl, alkynylaryl, substituted alkynylaryl, arylalkyl, substituted arylalkyl, arylalkenyl, substituted arylalkenyl, arylalkynyl, substituted arylalkynyl, poly(alkylene oxide), substituted poly(alkylene oxide), poly(alkylene sulfide), substituted poly(alkylene sulfide), poly(alkylene amine), substituted poly(alkylene amine), -OR, -SR or $-NR_2$, wherein each R is independently selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, alkylaryl, substituted alkylaryl, arylalkyl, substituted arylalkyl, poly(alkylene oxide), substituted poly(alkylene oxide), poly(alkylene sulfide), substituted poly(alkylene sulfide), poly(alkylene amine) or substituted poly(alkylene amine); with R_1

having in the range of 2 up to 15 carbon atoms being preferred;

R_2 is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, alkylaryl, substituted alkylaryl, alkenylaryl, substituted alkenylaryl, alkynylaryl, substituted alkynylaryl, arylalkyl, substituted arylalkyl, arylalkenyl, substituted arylalkenyl, arylalkynyl, substituted arylalkynyl, oxyalkyl, poly(alkylene oxide) or substituted poly(alkylene oxide); with R_2 having in the range of 1 up to about 15 carbon atoms being preferred;

R_3 is selected from hydrogen, hydroxy, halogen, alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl or substituted alkynyl; with R_3 having in the range of 0 up to about 6 carbon atoms being preferred;

R_4 is selected from hydrogen, formyl, acyl, lower alkyl or substituted lower alkyl; with R_4 having in the range of 0 up to about 4 carbon atoms being preferred;

R_5 is selected from hydrogen, hydroxy, lower alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl or halogen; with R_5 having in the range of 0 up to about 6 carbon atoms being preferred; and

R_6 is selected from hydrogen, hydroxy, lower alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl or halogen; with R_6 having in the range of 0 up to about 6 carbon atoms being preferred.

Those of skill in the art recognize that the core ring of structure I can be any one of a number of different aromatic or pseudo-aromatic structures, e.g., a benzene ring, a pyridine ring, a pyrazine, an oxazine, and the like.

As employed herein, "lower alkyl" refers to straight or branched chain alkyl groups having in the range of about 1 up to 4 carbon atoms; "alkyl" refers to straight or branched chain alkyl groups having in the range of about 1 up to 12 carbon atoms; "substituted alkyl" refers to alkyl groups further bearing one or more substituents such as hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide, heteroatom-containing cyclic moieties, substituted heteroatom-containing cyclic moieties, and the like.

As employed herein, "alkenyl" refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon double bond, and having in the range of about 2 up to 12 carbon atoms and "substituted alkenyl" refers to alkenyl groups further bearing one or more substituents as set forth above.

As employed herein, "alkynyl" refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon triple bond, and having in the range of about 2 up to 12 carbon atoms, and "substituted alkynyl" refers to alkynyl groups further bearing one or more substituents as set forth above.

As employed herein, "aryl" refers to aromatic groups having in the range of 6 up to 14 carbon atoms and "substituted aryl" refers to aryl groups further bearing one or more substituents as set forth above.

As employed herein, "alkylaryl" refers to alkyl-substituted aryl groups and "substituted alkylaryl" refers to alkylaryl groups further bearing one or more substituents as set forth above.

As employed herein, "alkenylaryl" refers to alkenyl-substituted aryl groups and "substituted alkenylaryl" refers to alkenylaryl groups further bearing one or more substituents as set forth above.

As employed herein, "alkynylaryl" refers to alkynyl-substituted aryl groups and "substituted alkynylaryl" refers to alkynylaryl groups further bearing one or more substituents as set forth above.

As employed herein, "arylalkyl" refers to aryl-substituted alkyl groups and "substituted arylalkyl" refers to arylalkyl groups further bearing one or more substituents as set forth above.

As employed herein, "arylalkenyl" refers to aryl-substituted alkenyl groups and "substituted arylalkenyl" refers to arylalkenyl groups further bearing one or more substituents as set forth above.

As employed herein, "arylalkynyl" refers to aryl-substituted alkynyl groups and "substituted arylalkynyl" refers to arylalkynyl groups further bearing one or more substituents as set forth above.

As employed herein, "poly(alkylene oxide)" refers to compounds having the general structure:

$$-[(CR'_2)_x-O]_y-H,$$

wherein each R' is independently selected from hydrogen or lower alkyl, x falls in the range of 1 up to about 4 and y

falls in the range of 2 up to about 8; "substituted poly(alkylene oxide)" refers to poly(alkylene oxide) groups further bearing one or more substituents as set forth above.

As employed herein, "poly(alkylene sulfide)" refers to compounds having the general structure:

$$-[(CR'_2)_x-S]_y-H,$$

wherein R', x and y are as defined above; "substituted poly(alkylene sulfide)" refers to poly(alkylene sulfide) groups further bearing one or more substituents as set forth above.

As employed herein, "poly(alkylene amine)" refers to compounds having the general structure:

$$-[(CR'_2)_x-N(R')]_y-H,$$

wherein R', x and y are as defined above; "substituted poly(alkylene amine)" refers to poly(alkylene amine) groups further bearing one or more substituents as set forth above.

As employed herein, "heteroatom-containing cyclic moiety" refers to cyclic (i.e., 5-, 6- or 7-membered ring-containing) groups containing one or more heteroatoms (e.g., N, O, S, or the like) as part of the ring structure, and having in the range of 1 up to about 14 carbon atoms; and "substituted heteroatom-containing cyclic moiety" refers to heterocyclic groups further bearing one or more substituents as set forth above. Examples of heteroatom-containing cyclic moieties include furans, thiophenes, pyrroles, pyrazoles, diazoles, triazoles, tetrazoles, dithioles, oxathioles, oxazoles, isoxazoles, thiazoles, isothiazoles, oxadiazoles, oxatriazoles, dioxazoles,

oxathiazoles, pyrans, pyrones, dioxins, pyridines, pyrimidines, pyrazines, pyridazines, piperazines, diazines, triazines, oxazines, isoxazines, oxathiazines, oxadiazines, morpholines, azepins, oxepins, thiopins, diazepins, benzothiazoles, thiazolidinediones, and the like.

As employed herein, "acyl" refers to alkyl-carbonyl species.

As employed herein, "halogen" or "halo" refers to fluoro substituents, chloro substituents, bromo substituents or iodo substituents.

In a presently preferred aspect of the present invention, "R_1" of Formula I is selected from:

$-Y_n-(CR"R")_m-Z$,

$-Y_n-(CR"R")_{m'}-O-(CR"R")_{m'}-Z$, or

$-Y_n-(CR"R")_{m"}-N(R''')-(CR"R")_{m"}-Z$,

wherein:

Y is -O- or -S-,

n is 0 or 1,

each R" is independently selected from hydrogen, lower alkyl, substituted lower alkyl, hydroxy, lower alkoxy, thioalkyl, halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl or sulfonamide,

R''' is selected from hydrogen, lower alkyl or substituted allkyl,

m falls in the range of 1 up to 15,

each m' falls independently in the range of 1 up to 8,

each m" falls independently in the range of 0 up to 12, and

Z is selected from a heteroatom-containing cyclic moiety, a substituted heteroatom-containing cyclic moiety,

cyano, nitro, amino, carbamate, $-OR^a$, wherein R^a is selected from H, alkyl, alkenyl, alkynyl, acyl or aryl; $-C(O)R^b$, wherein R^b is selected from H, alkyl, substituted alkyl, alkoxy, alkylamino, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, aryloxy, arylamino, alkylaryl, substituted alkylaryl, alkenylaryl, substituted alkenylaryl, alkynylaryl, substituted alkynylaryl, arylalkyl, substituted arylalkyl, arylalkenyl, substituted arylalkenyl, arylalkynyl, substituted arylalkynyl, heterocyclic, substituted heterocyclic or trifluoromethyl; $-CO_2R^c$, wherein R^c is selected from H, alkyl, alkenyl, alkynyl or aryl; $-SR^a$, $-S(O)R^a$, $-S(O)_2R^a$ or $-S(O)_2NHR^a$, wherein each R^a is as defined above, and the like.

It is presently preferred that Z be selected from heteroatom-containing cyclic moieties, with polyheteroatom-containing cyclic moieties being especially preferred. Those of skill in the art can readily identify numerous groups which fall within the definition of "heteroatom-containing cyclic moieties", as set forth herein. Especially preferred are polyheteroatom-containing cyclic moieties, e.g., pyrazoles, diazoles, triazoles, tetrazoles, dithioles, oxathioles, oxazoles, isoxazoles, thiazoles, isothiazoles, oxadiazoles, oxatriazoles, dioxazoles, oxathiazoles, pyridazines, piperazines, diazines, triazines, oxazines, isoxazines, oxathiazines, oxadiazines, morpholines, diazepins, thiazolidinediones, and the like.

Especially preferred compounds employed in the practice of the present invention are those wherein "R_1" of Formula I is

$$-Y_n-(CH_2)_x-Z$$

wherein:

Y is -O- or -S-,

n is 0 or 1,

x falls in the range of 2 up to 12; and

Z is a triazole, a tetrazole, an oxadiazole, an oxatriazole, a dioxazole, an oxathiazole, a triazine, an isoxazine, an oxathiazine, an oxadiazine, a thiazolidinedione, and the like.

A presently preferred species of R_1 is $-O-(CH_2)_4-$[tetrazoline].

In another preferred aspect of the present invention, "R_2" of Formula I is selected from methyl, ethyl, propyl, butyl, methoxy, ethoxy, propoxy, butoxy, and the like.

In yet another preferred aspect of the present invention, "R_3" of Formula I is selected from hydrogen, hydroxy, alkoxy, and the like.

In still another preferred aspect of the present invention, "R_4" of Formula I is selected from formyl, acyl, thiazolidenedione, alkyl-substituted thiazolidenedione, and the like.

In a further preferred aspect of the present invention, "R_5" of Formula I is hydrogen.

In a still further preferred aspect of the present invention, "R_6" of Formula I is hydrogen.

In yet another preferred aspect of the present invention, at least one of R_2, R_3, R_4, R_5 and R_6 (in addition to R_1) are not hydrogen. It is especially preferred that at least two of R_2, R_3, R_4, R_5 and R_6 (in addition to R_1) are not hydrogen. A plurality of substituents on the ring of structure I is especially preferred when the backbone of R_1 contains no greater than 6 atoms.

Presently preferred species contemplated for use in the practice of the present invention include compounds wherein:

R_1 is $-O-(CH_2)_4-$[tetrazoline],
R_2 is hydrogen or lower alkyl,
R_3 is hydroxy or alkoxy,
R_4 is acyl or thiazolidenedione; and
R_5 and R_6 are each hydrogen, as well as compounds wherein:

R_1 is $-O-(CH_2)_y-$thiazolidenedione, wherein y falls in the range of about 2 up to 8;
R_2 is hydrogen or lower alkyl,
R_3 is hydroxy or alkoxy,
R_4 is acyl or thiazolidenedione; and
R_5 and R_6 are each hydrogen.

The above-described compounds can be readily prepared using a variety of synthetic methods, as are well known by those of skill in the art. For example, many of the above-described compounds can be prepared chemically or enzymatically.

As employed herein, the term "modulate" refers to the ability of a modulator for a member of the steroid/thyroid superfamily to either directly (by binding to the receptor as a ligand) or indirectly (as a precursor

for a ligand or an inducer which promotes production of ligand from a precursor) induce expression of gene(s) maintained under hormone expression control, or to repress expression of gene(s) maintained under such control.

As employed herein, the phrase "processes mediated by PPARγ" refers to biological, physiological, endocrinological, and other bodily processes which are mediated by receptor or receptor combinations which are responsive to the PPAR-γ antagonists and partial-agonists described herein (e.g., cell differentiation to produce lipid-accumulating cells, regulation of insulin-sensitivity and blood glucose levels, especially as related to hypoglycemia/hyperinsulinism (resulting, for example, from abnormal pancreatic beta-cell function, insulin-secreting tumors and/or autoimmune hypoglycemia due to autoantibodies to insulin, the insulin receptor or autoantibodies that are stimulatory to pancreatic beta-cells), the formation of macrophages which lead to the development of atherosclerotic plaques, and the like). Modulation of such processes can be accomplished *in vitro* or *in vivo*. *In vivo* modulation can be carried out in a wide range of subjects, such as, for example, humans, rodents, sheep, pigs, cows, and the like.

PPAR-γ-selective antagonists or partial-agonists contemplated for use in the practice of the present invention can be employed for both *in vitro* and *in vivo* applications. For *in vivo* applications, the invention compounds can be incorporated into a pharmaceutically acceptable formulation for administration. Those of skill in the art can readily determine suitable dosage levels when compounds contemplated for use in the practice of the present invention are so used.

In accordance with another embodiment of the present invention, there is provided a method of screening

for antagonists of PPARγ receptor proteins, said method comprising

culturing test cells containing

(i) increasing concentrations of at least one compound whose ability to inhibit the transcription activation activity of PPARγ agonists is sought to be determined, and

(ii) optionally, at least one PPARγ agonist,

wherein said test cells contain

(i) exogenous DNA which expresses intact PPARγ or a modified form of PPARγ, wherein the modified form of PPARγ contains the DNA binding domain of GAL4, and

(ii) a PPRE or GAL4 response element, respectively, operatively linked to a reporter gene; and thereafter

assaying for evidence of transcription of said reporter gene in said cells as a function of the concentration of said compound in said culture medium, thereby indicating the ability of said compound to inhibit activation of transcription by PPARγ agonists.

Media employed for such culturing may include agonist for the receptor being tested, or the receptor may be constitutive (i.e., not require the presence of agonist for activation), or a fixed concentration of agonist can be added to the media employed for such testing.

The above-described assays of the present invention have low background and a broad dynamic range.

Thus, in accordance with the present invention, compound(s) which fall within the structure of Formula I can readily be tested for the ability to regulate the transcription-activating effects of peroxisome proliferator activated receptor-gamma (PPAR-γ). This can be carried out by assaying for changes in the level of reporter protein present as a result of contacting cells containing the receptor and reporter vector with test compound;

wherein the reporter vector comprises:

(a) a promoter that is operable in the cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein the reporter protein-encoding DNA segment is operatively linked to the promoter for transcription of the DNA segment, and

wherein the hormone response element is operatively linked to the promoter for activation thereof.

Hormone response elements contemplated for use in the practice of the present invention are composed of at least one direct repeat of two or more half sites separated by a spacer of one nucleotide. The spacer nucleotide can be selected from any one of A, C, G or T. Each half site of response elements contemplated for use in the practice of the invention comprises the sequence

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-. Response elements employed in the practice of the present invention can optionally be preceded by N_x, wherein x falls in the range of 0 up to 5.

Presently preferred response elements contain at least one copy (with one, two or three copies most common) of the minimal sequence:

AGGACA A AGGTCA (SEQ ID NO:4).

As noted above, the minimal sequence can optionally be flanked by additional residues, for example, as in the sequence:

GGACC AGGACA A AGGTCA CGTTC (SEQ ID NO:5).

In a preferred embodiment of the present invention, only the ligand binding domain of PPARγ is utilized, in combination with the DNA binding domain of GAL4 protein, for the identification of PPARγ ligands or ligand-precursors. This allows one to avoid possible background signal caused by the potential presence of endogenous PPAR isoforms in the host cells used for the assay.

The DNA binding domain of the yeast GAL4 protein comprises at least the first 74 amino acids thereof (see, for example, Keegan et al., Science 231:699-704 (1986)). Preferably, the first 90 or more amino acids of the GAL4 protein will be used, with the first 147 amino acid residues of yeast GAL4 being presently most preferred.

The GAL4 fragment employed in the practice of the present invention can be incorporated into any of a number

of sites within the PPARγ receptor protein. For example, the GAL4 DNA binding domain can be introduced at the amino terminus of the PPARγ receptor protein, or the GAL4 DNA binding domain can be substituted for the native DNA binding domain of the PPARγ receptor, or the GAL4 DNA binding domain can be introduced at the carboxy terminus of the PPARγ receptor protein, or at other positions as can readily be determined by those of skill in the art. Thus, for example, a modified receptor protein can be prepared which consists essentially of amino acid residues 1-147 of GAL4, plus the ligand binding domain of PPARγ (i.e., containing the ligand binding domain only of said receptor (i.e., residues 163-475 of SEQ ID NO:1), substantially absent the DNA binding domain and amino terminal domain thereof).

Identification methods according to the present invention involve the use of a functional bioassay system, wherein the modified receptor and a reporter plasmid are cultured in suitable host cells in the presence of test compound. Evidence of transcription (e.g., expression) of reporter gene is then monitored to determine the presence of an activated receptor-ligand complex. Accordingly, the functional bioassay system utilizes two plasmids: an "expression" plasmid and a "reporter" plasmid. The expression plasmid can be any plasmid which contains and is capable of expressing DNA encoding the desired form of PPARγ receptor protein (i.e., intact receptor or GAL4 chimeric receptor as described hereinabove), in a suitable host cell. The reporter plasmid can be any plasmid which contains an operative PPRE or GAL4 response element, as appropriate, functionally linked to an operative reporter gene.

Exemplary PPREs have been described in detail hereinabove. Exemplary GAL4 response elements are those containing the palindromic 17-mer:

5'-CGGAGGACTGTCCTCCG-3' (SEQ ID NO:6),

such as, for example, 17MX, as described by Webster et al., in Cell 52:169-178 (1988), as well as derivatives thereof. Additional examples of suitable response elements include those described by Hollenberg and Evans in Cell 55:899-906 (1988); or Webster et al. in Cell 54:199-207 (1988).

Exemplary reporter genes include chloramphenicol transferase (CAT), luciferase (LUC), beta-galactosidase (β-gal), and the like. Exemplary promoters include the simian virus (SV) promoter or modified form thereof (e.g., ΔSV), the thymidine kinase (TK) promoter, the mammary tumor virus (MTV) promoter or modified form thereof (e.g., ΔMTV), and the like [see, for example, Mangelsdorf et al., in Nature 345:224-229 (1990), Mangelsdorf et al., in Cell 66:555-561 (1991), and Berger et al., in J. Steroid Biochem. Molec. Biol. 41:733-738 (1992)]. The plasmids pGMCAT, pGHCAT, pTK-GAL$_p$3-LUC, ΔMTV-GAL$_p$3-LUC, ΔMTV-GAL$_p$3-CAT, and the like, are examples of reporter plasmids which contain an operative hormone responsive promoter/enhancer element functionally linked to an operative reporter gene, and can therefore be used in the above-described functional bioassay (see Example 2 for details on the preparation of these plasmids). In pGMCAT, the operative hormone responsive promoter/enhancer element is the MTV LTR; in pGHCAT it is the functional portion of the growth hormone promoter. In both pGMCAT and GHCAT the operative reporter gene is the bacterial gene for chloramphenicol acetyltransferase (CAT).

As used herein in the phrase "operative response element functionally linked to an operative reporter gene", the word "operative" means that the respective DNA sequences (represented by the terms "PPRE," "GAL4 response element" and "reporter gene") are operational, i.e., work for their intended purposes; the word "functionally" means

that after the two segments are linked, upon appropriate activation by a ligand-receptor complex, the reporter gene will be expressed as the result of the fact that the "PPRE" or "GAL4 response element" was "turned on" or otherwise activated.

In practicing the above-described functional bioassay, the expression plasmid and the reporter plasmid are co-transfected into suitable host cells. The transfected host cells are then cultured in the presence and absence of a test compound to determine if the test compound is able to produce activation of the promoter operatively linked to the PPRE or GAL4 response element of the reporter plasmid. Thereafter, the transfected and cultured host cells are monitored for induction (i.e., the presence) of the product of the reporter gene sequence.

Any cell line can be used as a suitable "host" for the functional bioassay contemplated for use in the practice of the present invention. Thus, in contrast to the requirements of prior art assay systems, when GAL4 chimerics are employed, there is no need to use receptor-negative cells in carrying out the invention process. Since the modified receptor employed in the practice of the present invention is the only species in the test cell which is capable of initiating transcription from a GAL4 response element, the expression of native receptor by the test cell does not contribute to background levels. Thus, the invention bioassay can be made to be very selective.

Cells contemplated for use in the practice of the present invention include transformed cells, non-transformed cells, neoplastic cells, primary cultures of different cell types, and the like. Exemplary cells which can be employed in the practice of the present invention include Schneider cells, CV-1 cells, HuTu80 cells, F9 cells, NTERA2 cells, NB4 cells, HL-60 cells, 293 cells,

Hela cells, yeast cells, and the like. Preferred host cells for use in the functional bioassay system are COS cells and CV-1 cells. COS-1 (referred to as COS) cells are monkey kidney cells that express SV40 T antigen (Tag); while CV-1 cells do not express SV40 Tag. The presence of Tag in the COS-1 derivative lines allows the introduced expression plasmid to replicate and provides a relative increase in the amount of receptor produced during the assay period. CV-1 cells are presently preferred because they are particularly convenient for gene transfer studies and provide a sensitive and well-described host cell system.

The above-described cells (or fractions thereof) are maintained under physiological conditions when contacted with physiologically active compound. "Physiological conditions" are readily understood by those of skill in the art to comprise an isotonic, aqueous nutrient medium at a temperature of about 37°C.

In accordance with yet another embodiment of the present invention, there is provided a method for treating obesity, said method comprising administering to a subject in need thereof an amount of a peroxisome proliferator activated receptor-gamma (PPAR-γ) antagonist effective to block cell differentiation to produce lipid-accumulating cells. As employed herein "treating" obesity embraces preventing as well as reversing obesity.

As employed here, "obesity" refers generally to individuals who are at least about 20-30% over the average weight for his/her age, sex and height. Technically, "obese" is defined, for males, as individuals whose body mass index is greater than 27.8 kg/m^2, and for females, as individuals whose body mass index is greater than 27.3 kg/m^2. Those of skill in the art readily recognize that the invention method is not limited to those who fall within

the above criteria. Indeed, the invention method can also be advantageously practiced by individuals who fall outside of these traditional criteria, for example, by those who may be prone to obesity.

Those of skill in the art recognize that there are numerous cell types which are capable of differentiation to produce "lipid-accumulating cells," such as, for example, mesenchymal cells (e.g., fibroblasts).

As employed herein, the phrase "amount... effective to block cell differentiation" refers to levels of compound sufficient to provide circulating concentrations high enough to effect activation of PPARγ. Such a concentration typically falls in the range of about 10 nM up to 2 μM; with concentrations in the range of about 100 nM up to 500 nM being preferred. Since the activity of different compounds which fall within the definition of structure **I** as set forth above may vary considerably, and since individual subjects may present a wide variation in severity of symptoms, it is up to the practitioner to determine a subject's response to treatment and vary the dosages accordingly.

In accordance with a particular embodiment of the present invention, compositions comprising at least one antagonist or partial-agonist of PPAR-γ (as described herein), and a pharmaceutically acceptable carrier are contemplated. Exemplary pharmaceutically acceptable carriers include carriers suitable for oral, intravenous, subcutaneous, intramuscular, intracutaneous, and the like administration. Administration in the form of creams, lotions, tablets, dispersible powders, granules, syrups, elixirs, sterile aqueous or non-aqueous solutions, suspensions or emulsions, and the like, is contemplated.

For the preparation of oral liquids, suitable carriers include emulsions, solutions, suspensions, syrups, and the like, optionally containing additives such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents, and the like.

For the preparation of fluids for parenteral administration, suitable carriers include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized, for example, by filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions. They can also be manufactured in the form of sterile water, or some other sterile injectable medium immediately before use.

In accordance with still another embodiment of the present invention, there is provided a method for modulating insulin-sensitivity and blood glucose levels in a subject, said method comprising administering to a subject in need of such treatment an amount of a peroxisome proliferator activated receptor-gamma (PPAR-γ) antagonist or partial-agonist effective to lower the blood glucose level of said subject.

As employed herein, the phrase "amount... effective to lower blood glucose levels" refers to levels of compound sufficient to provide circulating concentrations high enough to accomplish the desired effect. Such a concentration typically falls in the range of about 10 nM up to 2 μM; with concentrations in the range

of about 100 nM up to 500 nM being preferred. As noted previously, since the activity of different compounds which fall within the definition of structure I as set forth above may vary considerably, and since individual subjects may present a wide variation in severity of symptoms, it is up to the practitioner to determine a subject's response to treatment and vary the dosages accordingly.

The invention will now be described in greater detail by reference to the following non-limiting examples.

Example 1

Preparation of GAL4-receptor fusion proteins

A basic vector useful for the generation of GAL4-receptor fusion proteins is called pCMX-GAL4 (see SEQ ID NO:2). This vector encodes GAL4 DNA binding domain, followed by a polylinker sequence useful in the cloning. The parental expression vector pCMX has been described by Umesono et al., in Cell 65:1255-1266 (1991), and the GAL4 portion of pCMX-GAL4 is derived from plasmid pSG424, described by Sadowski and Ptashne, in Nucleic Acids Res. 17:7539 (1989).

In general, GAL4-receptor ligand binding domain fusions are prepared by taking advantage of mutant receptor cDNA clones, such as GR-RAR chimera [see, for example, Giguere et al., in Nature 330:624-629 (1987)]. These mutant receptor cDNAs encode common *Xho*I sites at the end of the DNA binding domain, as described by Giguere et al., supra. To do so, a new pCMX-GAL4 vector was prepared which encodes a compatible *Sal*I site in the polylinker sequence (there is an *Xho*I site in the GAL4 sequence):

*Sal*I site: G'TCGAC

*Xho*I site: C'TCGAG

This allows efficient transfer of the receptor ligand binding domain to GAL4 DNA binding domain. Through this

method, a number of chimeric species have been generated, including GAL4-PPARγ, containing residues 163-475 of PPARγ (see SEQ ID NO:1).

If mutants of the type referred to above are not available for the construction of GAL4-containing chimerics, one may simply look for any convenient restriction enzyme site within or downstream of the DNA binding domain of the receptor of interest (i.e., within about the first 30 amino acid residues downstream of the conserved Gly-Met residues of the DNA binding domain, i.e., within 30 residues of the last two residues shown in SEQ ID NO:1), and utilize the carboxy terminal sequences therefrom.

Example 2
Preparation of reporter constructs

Various reporter constructs are used in the examples which follow. They are prepared as follows:

TK-LUC: The MTV-LTR promoter sequence was removed from the MTV-LUC plasmid described by Hollenberg and Evans in Cell 55:899-906 (1988) by *Hin*dIII and *Xho*I digest, and cloned with the *Hin*dIII-*Xho*I fragment of the Herpes simplex virus thymidine kinase gene promoter (-105 to +51 with respect to the transcription start site, m, isolated from plasmid pBLCAT2, described by Luckow & Schutz in Nucleic Acids Res. 15:5490 (1987)) to generate parental construct TK-LUC.

pTK-PPRE3-LUC: Three copies of double-stranded peroxisome proliferator response element (PPRE) oligonucleotides (see SEQ ID NO:5) were cloned upstream of the TK promoter of TK-LUC at the *Sal*I site.

pTK-MH100x4-LUC: Four copies of double-stranded MH100 oligonucleotides, encoding a GAL4 binding site, were cloned upstream of the TK promoter of TK-LUC at the *Hin*dIII site.

CMX-βGAL: The coding sequence for the *E. coli* β-galactosidase gene was isolated from plasmid pCH110 [see Hall et al., J. Mol. Appl. Genet. 2:101-109 (1983)] by *Hin*dIII and *Bam*HI digest, and cloned into pCMX eucaryotic expression vector [see Umesono et al., supra].

Example 3
Screening assay for PPAR-γ antagonists

Effector plasmid, reporter plasmid, and β-galactosidase control plasmid are co-transfected into CV-1 cells at a ratio of about 1:3:5, using a liposome-mediated method, employing N-{2-(2,3)-dioleoyloxy)propyl-N,N,N-trimethyl ammonium methyl sulfate} (i.e., DOTAP, Boehringer Mannheim) according to the manufacturer's instructions in Dulbecco's modified Eagle's medium (DMEM) with 10% delipidated hormone-depleted fetal calf serum. After about 2-3 hours, the cells are washed with DMEM and agonist (200 nM BRL 49653) and/or an appropriate test compound (LY 171883; see Figure 1) is added to the media. After 24-48 hours of incubation, the cells are rinsed with phosphate buffered saline (pH 7.2) and lysed. Aliquots are assayed for luciferase and β-galactosidase activity. Luciferase activity is normalized to optical density units of β-galactosidase per minute of incubation.

Thus, CV-1 cells are co-transfected with CMX-GAL-PPARγ and pTK-MH100x4-LUC at a ratio of about 100 ng of receptor-encoding DNA per 10^5 cells. The usual amounts of DNA per 10^5 cells are 100 ng of CMX-GAL-PPARγ, 300 ng of pTK-MH100x4-LUC, and 500 ng of CMX-βGAL.

Typically, transfections are performed in triplicate. The plates are then incubated for 2-3 hours at 37°C.

The cells are washed with fresh medium. Fresh medium containing agonist (200 nM BRL 49653) and/or an appropriate test compound (LY 171883; see Figure 1) is added to the media. A solvent control is also performed. The cells are incubated at 37°C for 1-2 days.

The cells are rinsed twice with buffered saline solution. Subsequently, cells are lysed, *in situ*, by adding 200 μl of lysis buffer. After 30 minutes incubation at room temperature, 40 μl aliquots of cell lysate are transferred to 96-well plates for luciferase reporter gene assays and β-galactosidase transfection controls [see Heyman et al., Cell 68:397-406 (1992)].

The data are expressed as relative light units (RLUs) per O.D. unit of β-galactosidase per minute. The triplicates are averaged and plotted (see Figure 1) as relative reporter activity induced by agonist alone, antagonist alone, or combinations thereof. Review of the data presented in Figure 1 reveals that 2-hydroxy-3-propyl-4-[6-(tetrazole-5-yl)butoxy]acetophenone (i.e., LY 171883) is a functional antagonist of PPARγ.

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Evans, Ronald M.
Forman, Barry M.

(ii) TITLE OF INVENTION: MODULATORS OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR-GAMMA, AND METHODS FOR THE USE THEREOF

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Pretty, Schroeder, Brueggemann & Clark
(B) STREET: 444 South Flower Street, Suite 2000
(C) CITY: Los Angeles
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 90071

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/477,493
(B) FILING DATE: 07-JUN-1995
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Reiter, Stephen E.
(B) REGISTRATION NUMBER: 31,192
(C) REFERENCE/DOCKET NUMBER: P41 9958

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 619-546-1995
(B) TELEFAX: 619-546-9392

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2005 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 352..1776

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATCGAATCCC GCGCCCCAGG CGCTGCCGCT CTGAGTGCGA CGGGCCCCGC CTGGCCGGCC 60

GGAGGACGCG GAAGAAGAGA CCTGGGGCGC TGCCTGGGGT ATTGGGTCGC GCGCAGTGAG 120

GGGACCGAGT GTGACGACAA GGTGACCGGG CTGAGGGGAC GGGCTGAGGA GAAGTCACAC 180

TCTGACAGGA GCCTGTGAGA CCAACAGCCT GACGGGGTCT CGGTTGAGGG GACGCGGGCT 240

```
GAGAAGTCAC GTTCTGACAG GACTGTGTGA CAGACAAGAT TTGAAAGAAG CGGTGAACCA      300

CTGATATTCA GGACATTTTT AAAAACAAGA CTACCCTTTA CTGAAATTAC C ATG GTT       357
                                                          Met Val
                                                            1

GAC ACA GAG ATG CCA TTC TGG CCC ACC AAC TTC GGA ATC AGC TCT GTG         405
Asp Thr Glu Met Pro Phe Trp Pro Thr Asn Phe Gly Ile Ser Ser Val
      5                  10                  15

GAC CTC TCC GTG ATG GAA GAC CAC TCG CAT TCC TTT GAC ATC AAG CCC         453
Asp Leu Ser Val Met Glu Asp His Ser His Ser Phe Asp Ile Lys Pro
  20                  25                  30

TTT ACC ACA GTT GAT TTC TCC AGC ATT TCT GCT CCA CAC TAT GAA GAC         501
Phe Thr Thr Val Asp Phe Ser Ser Ile Ser Ala Pro His Tyr Glu Asp
35                  40                  45                  50

ATT CCA TTC ACA AGA GCT GAC CCA ATG GTT GCT GAT TAC AAA TAT GAC         549
Ile Pro Phe Thr Arg Ala Asp Pro Met Val Ala Asp Tyr Lys Tyr Asp
                  55                  60                  65

CTG AAG CTC CAA GAA TAC CAA AGT GCG ATC AAA GTA GAA CCT GCA TCT         597
Leu Lys Leu Gln Glu Tyr Gln Ser Ala Ile Lys Val Glu Pro Ala Ser
              70                  75                  80

CCA CCT TAT TAT TCT GAA AAG ACC CAG CTC TAC AAC AGG CCT CAT GAA         645
Pro Pro Tyr Tyr Ser Glu Lys Thr Gln Leu Tyr Asn Arg Pro His Glu
          85                  90                  95

GAA CCT TCT AAC TCC CTC ATG GCC ATT GAG TGC CGA GTC TGT GGG GAT         693
Glu Pro Ser Asn Ser Leu Met Ala Ile Glu Cys Arg Val Cys Gly Asp
      100                 105                 110

AAA GCA TCA GGC TTC CAC TAT GGA GTT CAT GCT TGT GAA GGA TGC AAG         741
Lys Ala Ser Gly Phe His Tyr Gly Val His Ala Cys Glu Gly Cys Lys
115                 120                 125                 130

GGT TTT TTC CGA AGA ACC ATC CGA TTG AAG CTT ATT TAT GAT AGG TGT         789
Gly Phe Phe Arg Arg Thr Ile Arg Leu Lys Leu Ile Tyr Asp Arg Cys
                  135                 140                 145

GAT CTT AAC TGC CGG ATC CAC AAA AAA AGT AGA AAT AAA TGT CAG TAC         837
Asp Leu Asn Cys Arg Ile His Lys Lys Ser Arg Asn Lys Cys Gln Tyr
              150                 155                 160

TGT CGG TTT CAG AAG TGC CTT GCT GTG GGG ATG TCT CAC AAT GCC ATC         885
Cys Arg Phe Gln Lys Cys Leu Ala Val Gly Met Ser His Asn Ala Ile
          165                 170                 175

AGG TTT GGG CGG ATG CCA CAG GCC GAG AAG GAG AAG CTG TTG GCG GAG         933
Arg Phe Gly Arg Met Pro Gln Ala Glu Lys Glu Lys Leu Leu Ala Glu
      180                 185                 190

ATC TCC AGT GAT ATC GAC CAG CTG AAC CCA GAG TCT GCT GAT CTG CGA         981
Ile Ser Ser Asp Ile Asp Gln Leu Asn Pro Glu Ser Ala Asp Leu Arg
195                 200                 205                 210

GCC CTG GCA AAG CAT TTG TAT GAC TCA TAC ATA AAG TCC TTC CCG CTG        1029
Ala Leu Ala Lys His Leu Tyr Asp Ser Tyr Ile Lys Ser Phe Pro Leu
                  215                 220                 225

ACC AAA GCC AAG GCG AGG GCG ATC TTG ACA GGA AAG ACA ACG GAC AAA        1077
Thr Lys Ala Lys Ala Arg Ala Ile Leu Thr Gly Lys Thr Thr Asp Lys
              230                 235                 240
```

```
TCA CCA TTT GTC ATC TAC GAC ATG AAT TCC TTA ATG ATG GGA GAA GAT      1125
Ser Pro Phe Val Ile Tyr Asp Met Asn Ser Leu Met Met Gly Glu Asp
        245                 250                 255

AAA ATC AAG TTC AAA CAT ATC ACC CCC CTG CAG GAG CAG AGC AAA GAG      1173
Lys Ile Lys Phe Lys His Ile Thr Pro Leu Gln Glu Gln Ser Lys Glu
    260                 265                 270

GTG GCC ATC CGA ATT TTT CAA GGG TGC CAG TTT CGA TCC GTA GAA GCC      1221
Val Ala Ile Arg Ile Phe Gln Gly Cys Gln Phe Arg Ser Val Glu Ala
275                 280                 285                 290

GTG CAA GAG ATC ACA GAG TAT GCC AAA AAT ATC CCT GGT TTC ATT AAC      1269
Val Gln Glu Ile Thr Glu Tyr Ala Lys Asn Ile Pro Gly Phe Ile Asn
                295                 300                 305

CTT GAT TTG AAT GAC CAA GTG ACT CTG CTC AAG TAT GGT GTC CAT GAG      1317
Leu Asp Leu Asn Asp Gln Val Thr Leu Leu Lys Tyr Gly Val His Glu
            310                 315                 320

ATC ATC TAC ACG ATG CTG GCC TCC CTG ATG AAT AAA GAT GGA GTC CTC      1365
Ile Ile Tyr Thr Met Leu Ala Ser Leu Met Asn Lys Asp Gly Val Leu
        325                 330                 335

ATC TCA GAG GGC CAA GGA TTC ATG ACC AGG GAG TTC CTC AAA AGC CTG      1413
Ile Ser Glu Gly Gln Gly Phe Met Thr Arg Glu Phe Leu Lys Ser Leu
    340                 345                 350

CGG AAG CCC TTT GGT GAC TTT ATG GAG CCT AAG TTT GAG TTT GCT GTG      1461
Arg Lys Pro Phe Gly Asp Phe Met Glu Pro Lys Phe Glu Phe Ala Val
355                 360                 365                 370

AAG TTC AAT GCA CTG GAA TTA GAT GAC AGT GAC TTG GCT ATA TTT ATA      1509
Lys Phe Asn Ala Leu Glu Leu Asp Asp Ser Asp Leu Ala Ile Phe Ile
                375                 380                 385

GCT GTC ATT ATT CTC AGT GGA GAC CGC CCA GGC TTG CTG AAC GTG AAG      1557
Ala Val Ile Ile Leu Ser Gly Asp Arg Pro Gly Leu Leu Asn Val Lys
            390                 395                 400

CCC ATC GAG GAC ATC CAA GAC AAC CTG CTG CAG GCC CTG GAA CTG CAG      1605
Pro Ile Glu Asp Ile Gln Asp Asn Leu Leu Gln Ala Leu Glu Leu Gln
        405                 410                 415

CTC AAG CTG AAT CAC CCA GAG TCC TCT CAG CTG TTC GCC AAG GTG CTC      1653
Leu Lys Leu Asn His Pro Glu Ser Ser Gln Leu Phe Ala Lys Val Leu
    420                 425                 430

CAG AAG ATG ACA GAC CTC AGG CAG ATC GTC ACA GAG CAC GTG CAG CTA      1701
Gln Lys Met Thr Asp Leu Arg Gln Ile Val Thr Glu His Val Gln Leu
435                 440                 445                 450

CTG CAT GTG ATC AAG AAG ACA GAG ACA GAC ATG AGC CTT CAC CCC CTG      1749
Leu His Val Ile Lys Lys Thr Glu Thr Asp Met Ser Leu His Pro Leu
                455                 460                 465

CTC CAG GAG ATC TAC AAG GAC TTG TAT TAGCAGGAAA GTCCCACCCG            1796
Leu Gln Glu Ile Tyr Lys Asp Leu Tyr
            470                 475

CTGACAACGT GTTCCTTCTA TTGATTGCAC TATTATTTTG AGGGAAAAAA ATCTGACACC    1856

TAAGAAATTT ACTGTGAAAA AGCATTTAAA AACAAAAAGT TTTAGAACAT GATCTATTTT    1916

ATGCATATTG TTTATAAAGA TACATTTACA ATTTACTTTT AATATTAAAA ATTACCACAT    1976

TATAAAAAAA AAAAAAAAAA AGGAATTCC                                      2005
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 546 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 35..544

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
GGGAGACCCA AGCTTGAAGC AAGCCTCCTG AAAG ATG AAG CTA CTG TCT TCT          52
                                       Met Lys Leu Leu Ser Ser
                                         1               5

ATC GAA CAA GCA TGC GAT ATT TGC CGA CTT AAA AAG CTC AAG TGC TCC        100
Ile Glu Gln Ala Cys Asp Ile Cys Arg Leu Lys Lys Leu Lys Cys Ser
              10                  15                  20

AAA GAA AAA CCG AAG TGC GCC AAG TGT CTG AAG AAC AAC TGG GAG TGT        148
Lys Glu Lys Pro Lys Cys Ala Lys Cys Leu Lys Asn Asn Trp Glu Cys
          25                  30                  35

CGC TAC TCT CCC AAA ACC AAA AGG TCT CCG CTG ACT AGG GCA CAT CTG        196
Arg Tyr Ser Pro Lys Thr Lys Arg Ser Pro Leu Thr Arg Ala His Leu
      40                  45                  50

ACA GAA GTG GAA TCA AGG CTA GAA AGA CTG GAA CAG CTA TTT CTA CTG        244
Thr Glu Val Glu Ser Arg Leu Glu Arg Leu Glu Gln Leu Phe Leu Leu
 55                  60                  65                  70

ATT TTT CCT CGA GAA GAC CTT GAC ATG ATT TTG AAA ATG GAT TCT TTA        292
Ile Phe Pro Arg Glu Asp Leu Asp Met Ile Leu Lys Met Asp Ser Leu
                  75                  80                  85

CAG GAT ATA AAA GCA TTG TTA ACA GGA TTA TTT GTA CAA GAT AAT GTG        340
Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu Phe Val Gln Asp Asn Val
              90                  95                 100

AAT AAA GAT GCC GTC ACA GAT AGA TTG GCT TCA GTG GAG ACT GAT ATG        388
Asn Lys Asp Ala Val Thr Asp Arg Leu Ala Ser Val Glu Thr Asp Met
          105                 110                 115

CCT CTA ACA TTG AGA CAG CAT AGA ATA AGT GCG ACA TCA TCA TCG GAA        436
Pro Leu Thr Leu Arg Gln His Arg Ile Ser Ala Thr Ser Ser Ser Glu
      120                 125                 130

GAG AGT AGT AAC AAA GGT CAA AGA CAG TTG ACT GTA TCG CCG GAA TTC        484
Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu Thr Val Ser Pro Glu Phe
135                 140                 145                 150

CCG GGG ATC CGT CGA CGG TAC CAG ATA TCA GGA TCC TGG CCA GCT AGC        532
Pro Gly Ile Arg Arg Arg Tyr Gln Ile Ser Gly Ser Trp Pro Ala Ser
                  155                 160                 165

TAG GTA GCT AGA GG                                                     546
 *  Val Ala Arg
              170
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 170 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Lys Leu Leu Ser Ser Ile Glu Gln Ala Cys Asp Ile Cys Arg Leu
 1               5                  10                  15

Lys Lys Leu Lys Cys Ser Lys Glu Lys Pro Lys Cys Ala Lys Cys Leu
            20                  25                  30

Lys Asn Asn Trp Glu Cys Arg Tyr Ser Pro Lys Thr Lys Arg Ser Pro
        35                  40                  45

Leu Thr Arg Ala His Leu Thr Glu Val Glu Ser Arg Leu Glu Arg Leu
    50                  55                  60

Glu Gln Leu Phe Leu Leu Ile Phe Pro Arg Glu Asp Leu Asp Met Ile
65                  70                  75                  80

Leu Lys Met Asp Ser Leu Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu
                85                  90                  95

Phe Val Gln Asp Asn Val Asn Lys Asp Ala Val Thr Asp Arg Leu Ala
            100                 105                 110

Ser Val Glu Thr Asp Met Pro Leu Thr Leu Arg Gln His Arg Ile Ser
        115                 120                 125

Ala Thr Ser Ser Ser Glu Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu
    130                 135                 140

Thr Val Ser Pro Glu Phe Pro Gly Ile Arg Arg Arg Tyr Gln Ile Ser
145                 150                 155                 160

Gly Ser Trp Pro Ala Ser  *  Val Ala Arg
                165                 170
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AGGACAAAGG TCA 13

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGACCAGGAC AAAGGTCACG TTC 23

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

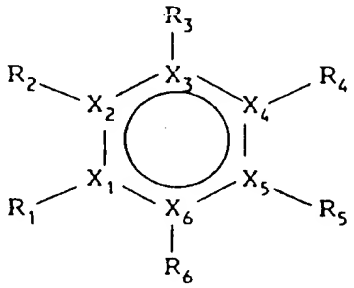
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGGAGGACTG TCCTCCG 17

That which is claimed is:

1. A method for modulating process(es) mediated by peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising conducting said process(es) in the presence of at least one antagonist or partial-agonist of PPAR-γ.

2. A method according to Claim 1, wherein said antagonist or partial-agonist of PPAR-γ has the structure I:



(I)

wherein:

each of X_1, X_2, X_3, X_4, X_5 and X_6 is independently selected from carbon, nitrogen, oxygen or sulfur, with the proviso that at least three of the atoms forming the ring are carbon,

R_1 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, alkylaryl, substituted alkylaryl, alkenylaryl, substituted alkenylaryl, alkynylaryl, substituted alkynylaryl, arylalkyl, substituted arylalkyl, arylalkenyl, substituted arylalkenyl, arylalkynyl, substituted arylalkynyl, poly(alkylene oxide), substituted poly(alkylene oxide), poly(alkylene sulfide), substituted poly(alkylene

sulfide), poly(alkylene amine), substituted poly(alkylene amine), -OR, -SR, $-NR_2$, wherein each R is independently selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, alkylaryl, substituted alkylaryl, arylalkyl, substituted arylalkyl, poly(alkylene oxide), substituted poly(alkylene oxide), poly(alkylene sulfide), substituted poly(alkylene sulfide), poly(alkylene amine) or substituted poly(alkylene amine);

R_2 is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, alkylaryl, substituted alkylaryl, alkenylaryl, substituted alkenylaryl, alkynylaryl, substituted alkynylaryl, arylalkyl, substituted arylalkyl, arylalkenyl, substituted arylalkenyl, arylalkynyl, substituted arylalkynyl, oxyalkyl, poly(alkylene oxide) or substituted poly(alkylene oxide);

R_3 is selected from hydrogen, hydroxy, halogen, alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl or substituted alkynyl;

R_4 is selected from hydrogen, formyl, acyl, lower alkyl or substituted lower alkyl;

R_5 is selected from hydrogen, hydroxy, lower alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl or halogen; and

R_6 is selected from hydrogen, hydroxy, lower alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl or halogen.

3. A method according to claim 2 wherein R_1 of Formula I is selected from:

$-Y_n-(CR"R")_m-Z$,

$-Y_n-(CR"R")_{m'}-O-C(R"R")_{m"}-Z$, or

$-Y_n-(CR"R")_{m'}-N(R''')-CR"R")_{m"}-Z$,

wherein:

Y is -O- or -S-,

n is 0 or 1,

each R" is independently selected from hydrogen, lower alkyl, substituted lower alkyl, hydroxy, lower alkoxy, thioalkyl, halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl or sulfonamide,

R''' is selected from hydrogen, lower alkyl or substituted alkyl,

m falls in the range of 1 up to 15,

each m' falls independently in the range of 1 up to 8,

each m" falls independently in the range of 0 up to 12, and

Z is a heteroatom-containing cyclic moiety, a substituted heteroatom-containing cyclic moiety, cyano, nitro, amino, carbamate, $-OR^a$, wherein R^a is selected from H, alkyl, alkenyl, alkynyl, acyl or aryl; $-C(O)R^b$, wherein R^b is selected from H, alkyl, substituted alkyl, alkoxy, alkylamino, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, aryloxy, arylamino, alkylaryl, substituted alkylaryl, arylalkyl, substituted arylalkyl, alkenylaryl, substituted alkenylaryl, alkynylaryl, substituted alkynylaryl, arylalkyl,

substituted arylalkyl, arylalkenyl, substituted arylalkenyl, arylalkynyl, substituted arylalkynyl, heterocyclic, substituted heterocyclic or trifluoromethyl; $-CO_2R^c$, wherein R^c is selected from H, alkyl, alkenyl or alkynyl; $-SR^a$, $-S(O)R^a$, $-S(O)_2R^a$ or $-S(O)_2NHR^a$, wherein each R^a is as defined above.

4. A method according to claim 3 wherein Z is a heteroatom-containing cyclic moiety or a substituted heteroatom-containing cyclic moiety.

5. A method according to claim 3 wherein Z is a polyheteroatom-containing cyclic moiety or a substituted polyheteroatom-containing cyclic moiety.

6. A method according to claim 3 wherein Z is a furan, thiophene, pyrrole, pyrazole, diazole, triazole, tetrazole, dithiole, oxathiole, oxazole, isoxazole, thiazole, isothiazole, oxadiazole, oxatriazole, dioxazole, oxathiazole, pyran, pyrone, dioxin, pyridine, pyrimidine, pyrazine, pyridazine, piperazine, diazine, triazine, oxazine, isoxazine, oxathiazine, oxadiazine, morpholino, azepin, oxepin, thiopin, diazepin, benzothiazole or a thiazolidinedione.

7. A method according to claim 3 wherein Z is a pyrazole, diazole, triazole, tetrazole, dithiole, oxathiole, oxazole, isoxazole, thiazole, isothiazole, oxadiazole, oxatriazole, dioxazole, oxathiazole, pyridazine, piperazine, diazine, triazine, oxazine, isoxazine, oxathiazine, oxadiazine, morpholine, diazepin or a thiazolidinedione.

8. A method according to claim 3 wherein R_1 of Formula I has the structure:

$$-Y_n-(CH_2)_x-Z$$

wherein:

Y is -O- or -S-,

n is 0 or 1,

x falls in the range of 2 up to 12; and

Z is a triazole, tetrazole, oxadiazole, oxatriazole, dioxazole, oxathiazole, triazine, isoxazine, oxathiazine, oxadiazine or a thiazolidinedione.

9. A method according to claim 2 wherein R_1 is $-O-(CH_2)_4-$[tetrazoline].

10. A method according to claim 2 wherein R_2 of Formula I is selected from methyl, ethyl, propyl, butyl, methoxy, ethoxy, propoxy or butoxy.

11. A method according to claim 2 wherein R_3 of Formula I is selected from hydrogen, hydroxy or alkoxy.

12. A method according to claim 2 wherein R_4 of Formula I is selected from formyl, acyl, thiazolidenediones or alkyl-substituted thiazolidenediones.

13. A method according to claim 2 wherein R_5 of Formula I is hydrogen.

14. A method according to claim 2 wherein R_6 of Formula I is hydrogen.

15. A method according to claim 1, wherein at least one of R_2, R_3, R_4, R_5 and R_6 are not hydrogen.

16. A method according to claim 1, wherein at least two of R_2, R_3, R_4, R_5 and R_6 are not hydrogen.

17. A method according to claim 16 wherein the backbone of R_1 contains no greater than 6 atoms.

18. A method according to claim 2 wherein:
R_1 is $-O-(CH_2)_4-$[tetrazoline],
R_2 is hydrogen or lower alkyl,
R_3 is hydroxy or alkoxy,
R_4 is acyl or thiazolidenedione; and
R_5 and R_6 are each hydrogen.

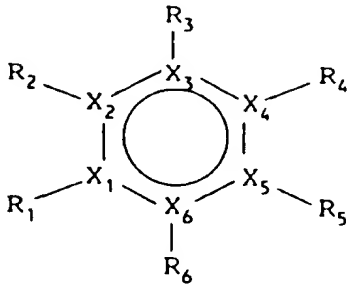
19. A method according to claim 2 wherein:
R_1 is $-O-(CH_2)_y$-thiazolidenedione, wherein y falls in the range of about 2 up to 8;
R_2 is hydrogen or lower alkyl,
R_3 is hydroxy or alkoxy,
R_4 is acyl or thiazolidenedione; and
R_5 and R_6 are each hydrogen.

20. A method according to claim 1 wherein said process mediated by PPAR-γ is cell differentiation to produce lipid-accumulating cells.

21. A method according to claim 1 wherein the process(es) mediated by PPAR-γ are insulin-sensitivity and blood glucose levels of the recipient.

22. A method for treating obesity, said method comprising administering to a subject in need of such treatment an amount of a peroxisome proliferator activated receptor-gamma (PPAR-γ) antagonist or partial-agonist effective to block cell differentiation to produce lipid-accumulating cells.

23. A method according to Claim 22, wherein said antagonist or partial-agonist of PPAR-γ has the structure I:



(I)

wherein:

each of X_1, X_2, X_3, X_4, X_5 and X_6 is independently selected from carbon, nitrogen, oxygen or sulfur, with the proviso that at least three of the atoms forming the ring are carbon,

R_1 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, alkylaryl, substituted alkylaryl, alkenylaryl, substituted alkenylaryl, alkynylaryl, substituted alkynylaryl, arylalkyl, substituted arylalkyl, arylalkenyl, substituted arylalkenyl, arylalkynyl, substituted arylalkynyl, poly(alkylene oxide), substituted poly(alkylene oxide), poly(alkylene sulfide), substituted poly(alkylene sulfide), poly(alkylene amine), substituted poly(alkylene amine), -OR, -SR, $-NR_2$, wherein each R is independently selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, alkylaryl, substituted alkylaryl, arylalkyl, substituted arylalkyl, poly(alkylene oxide),

substituted poly(alkylene oxide), poly(alkylene sulfide), substituted poly(alkylene sulfide), poly(alkylene amine) or substituted poly(alkylene amine);

R_2 is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, alkylaryl, substituted alkylaryl, alkenylaryl, substituted alkenylaryl, alkynylaryl, substituted alkynylaryl, arylalkyl, substituted arylalkyl, arylalkenyl, substituted arylalkenyl, arylalkynyl, substituted arylalkynyl, oxyalkyl, poly(alkylene oxide) or substituted poly(alkylene oxide);

R_3 is selected from hydroxy, halogen, alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl or substituted alkynyl;

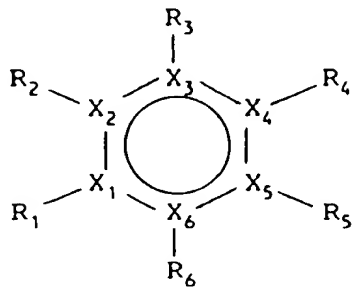
R_4 is selected from hydrogen, formyl, acyl, lower alkyl or substituted lower alkyl;

R_5 is selected from hydrogen, hydroxy, lower alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl or halogen; and

R_6 is selected from hydrogen, hydroxy, lower alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl or halogen.

24. A method for modulating insulin-sensitivity and blood glucose levels in a subject, said method comprising administering to a subject in need of such treatment an amount of a peroxisome proliferator activated receptor-gamma (PPAR-γ) antagonist or partial-agonist effective to lower the blood glucose level of said subject.

25. A composition comprising a compound having the structure I and a pharmaceutically acceptable carrier therefor, wherein structure I is as follows:



(I)

wherein:

each of X_1, X_2, X_3, X_4, X_5 and X_6 is independently selected from carbon, nitrogen, oxygen or sulfur, with the proviso that at least three of the atoms forming the ring are carbon,

R_1 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, alkylaryl, substituted alkylaryl, alkenylaryl, substituted alkenylaryl, alkynylaryl, substituted alkynylaryl, arylalkyl, substituted arylalkyl, arylalkenyl, substituted arylalkenyl, arylalkynyl, substituted arylalkynyl, poly(alkylene oxide), substituted poly(alkylene oxide), poly(alkylene sulfide), substituted poly(alkylene sulfide), poly(alkylene amine), substituted poly(alkylene amine), -OR, -SR, $-NR_2$, wherein each R is independently selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, alkylaryl, substituted alkylaryl, arylalkyl, substituted arylalkyl, poly(alkylene oxide),

substituted poly(alkylene oxide), poly(alkylene sulfide), substituted poly(alkylene sulfide), poly(alkylene amine) or substituted poly(alkylene amine);

R_2 is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, alkylaryl, substituted alkylaryl, alkenylaryl, substituted alkenylaryl, alkynylaryl, substituted alkynylaryl, arylalkyl, substituted arylalkyl, arylalkenyl, substituted arylalkenyl, arylalkynyl, substituted arylalkynyl, oxyalkyl, poly(alkylene oxide) or substituted poly(alkylene oxide);

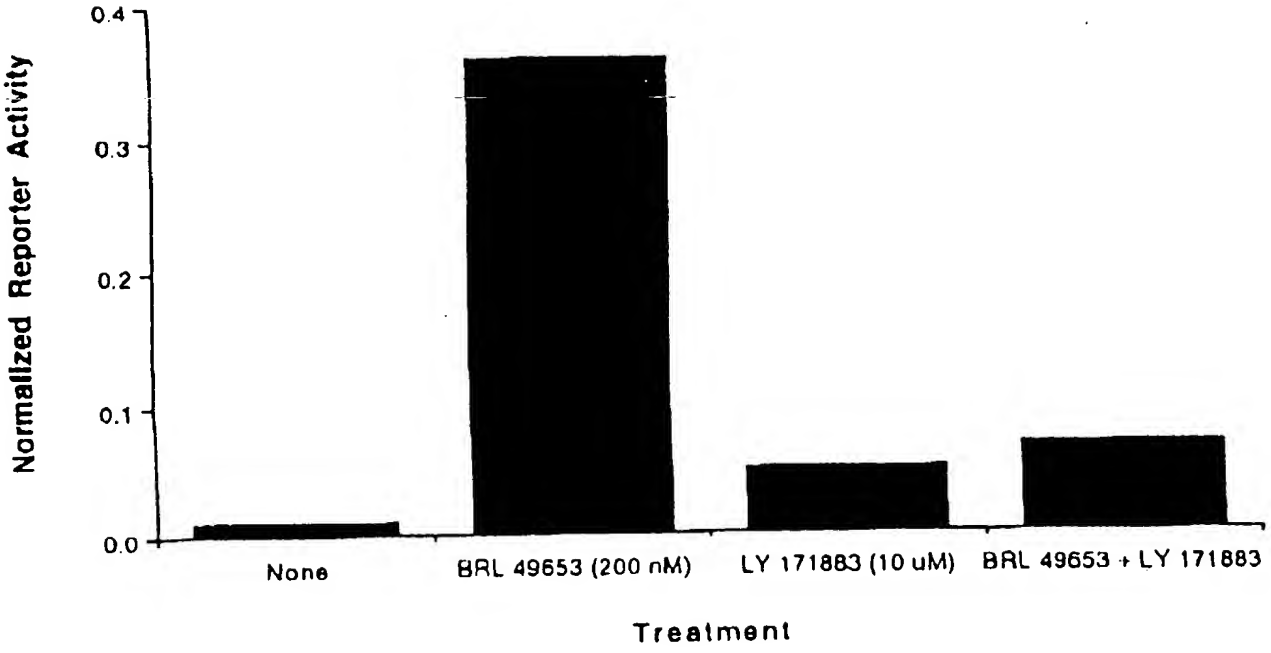
R_3 is selected from hydrogen, hydroxy, halogen, alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl or substituted alkynyl;

R_4 is selected from hydrogen, formyl, acyl, lower alkyl or substituted lower alkyl;

R_5 is selected from hydrogen, hydroxy, lower alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl or halogen; and

R_6 is selected from hydrogen, hydroxy, lower alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl or halogen.

FIGURE 1


Normalized Reporter Activity
0.4
0.3
0.2
0.1
0.0
None
BRL 49653 (200 nM)
LY 171883 (10 uM)
BRL 49653 + LY 171883
Treatment